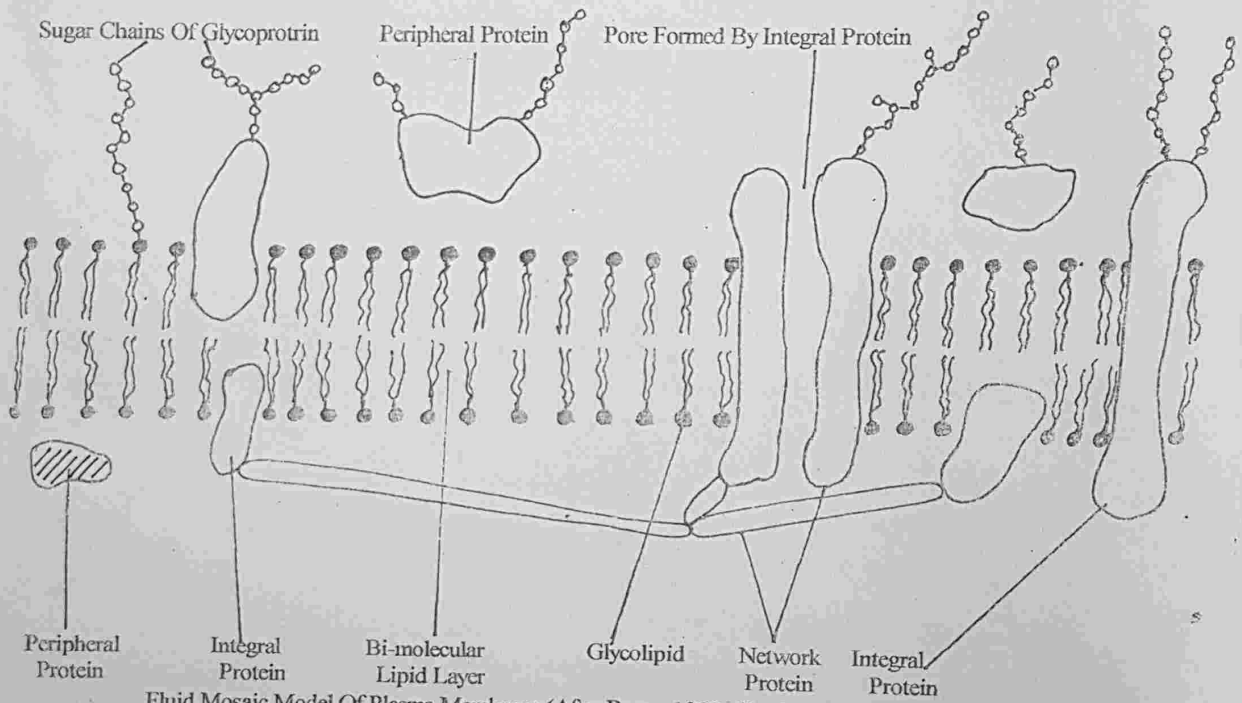
BIOLOGICAL MEMBRANE & TRANSPORT

Fluid Mosaic Model: S.J. Singer & G.L. Nicolson (1972) suggested the widely accepted fluid mosaic model of biological membranes. According to this model the plasma membrane contains a bimolecular lipid layer, both surface of which are interrupted by protein molecules. Proteins occur in the form of globular molecules & they are dotted about here & there in a mosaic pattern. Some proteins are attached at the polar surface of the lipid (i.e. the extrinsic protein) either partially penetrate the bi-layer or span the membrane entirely to stick out on both sides (called trans-membrane proteins). Further, the peripheral proteins & those parts of the integral proteins that stick on the outer surface (i.e. ectoproteins) frequently contain chains of sugar or oligosaccharide (i.e. they are glycoproteins). Likewise, some lipids of outer surface are glycolipids.

The fluid mosaic membrane is thought to be a far less rigid than was originally supposed. In fact, experiments its viscosity suggest that it is a fluid consisting rather like the oil, & that there is a considerable sideways movement of the lipid & protein molecules within it. On account of its fluidity & the mosaic arrangement of protein molecules, this model of membrane structure is known as the "Fluid Mosaic Model" (i.e. it describe both properties & organization f the membrane). The fluid mosaic model is found to be applied to all biological membrane in general, & it is seen as a dynamic ever-changing structure. The proteins are present not to give it strength, but to serve as enzymes catalyzing chemical reactions within the membrane & as pumps moving things across it.



Fluid Mosaic Model Of Plasma Membrane (After Berns, 1983; Sheeler & Biarchi,1987)

Mode Of Transport Across Plasma Membrane: The plasma membrane acts as a semi-permeable barrier between the cell & the extra-cellular environment. This permeability is highly selective. The selective permeability of the plasma membrane allows the cell to maintain a constant internal environment (Homeostasis).

Transport across the membrane may be passive or active. It may occur via the phospholipid bi-layer or by the help of specific integral membrane proteins, called permease or transport proteins.

Passive Transport: It is a type of diffusion in which an ion or molecule crossing a membrane moves down its electrochemical gradient, without consumption of energy. It is of following 3 types:

1. Osmosis: The plasma membrane is permeable to water molecules. The to & fro movement of water molecules through the plasma membrane occurs due to the differences in the concentration of the solute on either its sides. The process by which the water molecules pass through a membrane from a region of higher water concentration to a region of lower is known as OSMOSIS (Gr. Osmos = Pushing). The process in which the water molecules enter into the cell is known as

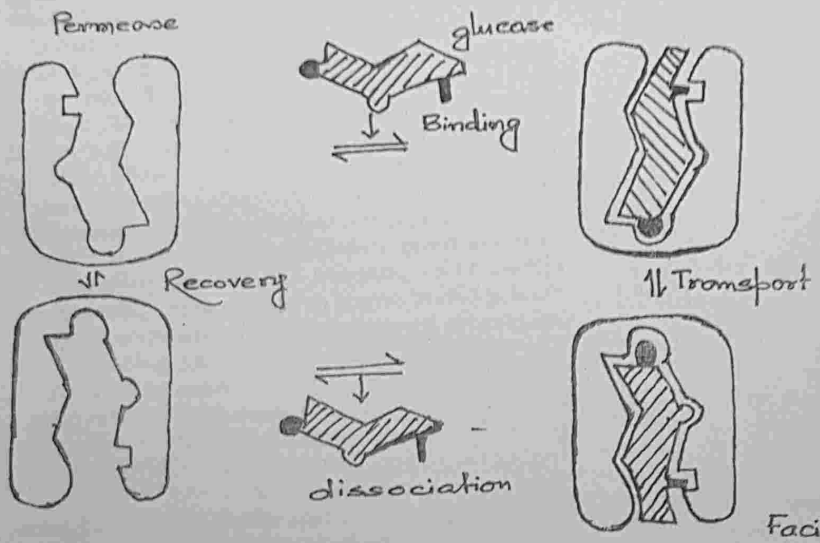
end-osmosis, while the reverse is known as ex-osmosis. In plant cells due to excessive ex-osmosis the cytoplasm along with the plasma membrane shrinks away from the cell wall, called PLASMOLYSIS (Gr. Plasma = molded).

2. Simple Diffusion: In simple diffusion transport across the membrane take place unaided i.e. molecule of gases oxygen & carbon-dioxide & small molecules (e.g. ethanol) enter the cell by crossing the plasma membrane without the help of any permease. During simple diffusion a small molecule in aqueous solution dissolves into the phospholipid bi-layer, crosses it & then dissolves into the aqueous solution on the opposite side. There is little specificity to the process. The relative rate of diffusion of the molecule across the phospholipid bi-layer will be proportional to the concentration gradient across the membrane.

3. Facilitated Diffusion: This is a special type of passive transport in which ions or molecules cross the membrane rapidly because specific permease in the membrane facilitated their crossing. Like the simple diffusion the facilitated diffusion does not require any metabolic energy & occurs only in the direction of concentration gradient. It is characterized by the following features: a) The rate of transport of the molecule across the membrane is far greater than would be expected from the simple diffusion.

b) It is a specific process ; each facilitated diffusion protein (called protein channel) transports only a single species of ion or molecule.

c) There is a maximum rate of transport i.e. when the concentration gradient of molecules across the membrane is low, in a corresponding increase in the rate of transport. It is believed that transport proteins form the channels through the membrane that permit certain ions or molecules to pass across the latter.

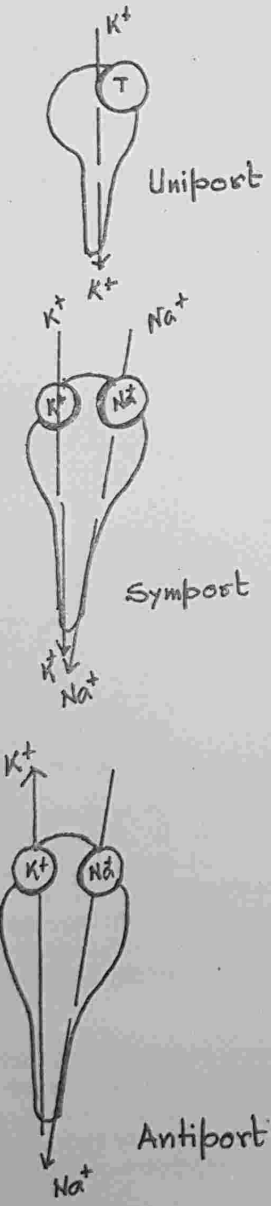


Facilited Diffusion.

Example of facilitated diffusion: Ionic transport through charged pores- Nerve conduction is propagated along the axonal membrane by action potential which regulates opening & closing of 2 main types of ion channels (i.e. channel proteins with water filled pores): Na+ channels (voltage gated Na+ channels) & K+ channels (K+ leak channels). At the point of stimulation there is a sudden & several 100 folds increase in permeability to Na+, which reaches its peak in 0.1 milliseconds (i.e. the membrane potential may depolarize from –90mV & overshoot to +50mV). At the end of the period, the membrane again becomes essentially impermeable to Na+, but the K+ permeability increases & this ion leaks out of the cell, re-polarizing the nerve fiber. During the rising phase of the spike, Na+ enters through the Na+ channels & in descending phase K+ is extruded through the K+ channels.

Active Transport: Active transport uses specific transport proteins, called pumps, which use metabolic energy (ATP) to moves ions or molecules against their concentration gradient. E.g. in both vertebrates & invertebrates the concentration of Na+ ion is about to 20 times higher in the blood than with in the cell. The concentration of the K+ ion is the reverse, generally 20-10 times higher inside the cell. Such a low Na+ concentration inside the cell is maintained by the Na-K pump. There are different types of for the different types of ions or molecules such as Ca pump, proton pump etc.

Examples- 1. Na+-K+-ATPase: It is an ion pump or cation exchange pump, which is driven by energy of 1 ATP molecule to export 3 Na+ ions outside the cell in exchange of 2 K+ ions inside the cell. Electrical organ of the eels are fond to be very rich in this enzyme or pump. Na+-K+-ATPase is a trans-membrane protein, which is a dimmer having 2 subunits : 1 smaller subunit (glycoprotein of 50,000 daltons M.W.) having an unknown function & another larger subunit having 1,20,000 daltons M.W. The large subunit of Na+-K+-ATPase perform the actual function of cation transport. It has 3 sites on its extra-cytoplasmic surface, 2 sites for K+ ions & 1 site for the inhibitor ovabain. On its cytosolic side, the large subunit contains 3 sites for Na+ ions & also has 1 catalytic site for ATP molecule. It is believed that the hydrolysis of 1 ATP molecule somehow drives conformational changes in the Na+-K+-ATPase that allows the pump to transport 3 Na+ ions out & 2 K+ inside the cell.

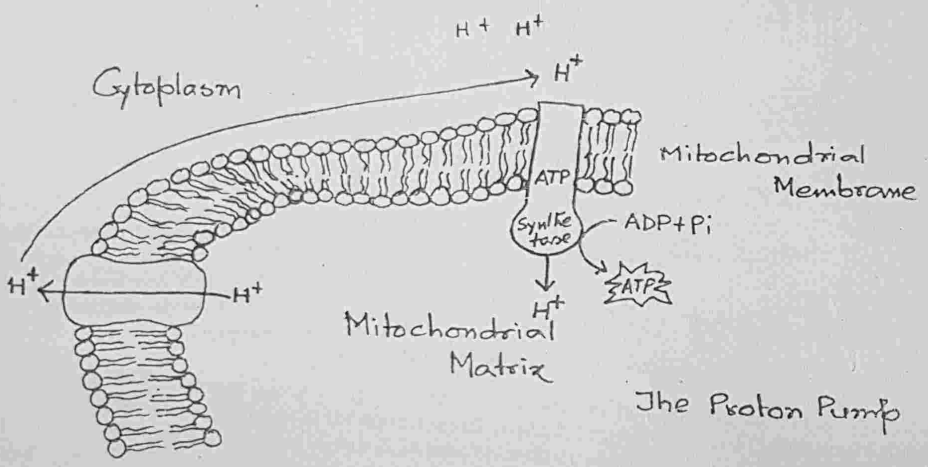


2. Ca^{+2}-ATPase: Ca^{+2} pump pumps Ca^{+2} out of the cytosol, maintaining a low concentration of it inside the cytosol. In some types of cells such as erythrocytes, the Ca^{+2} pumps are located in the plasma membrane & function to transport Ca^{+2} ions out of the cell. In contrust, in muscles cells Ca^{+2} ion pumps are located in the membrane of ER or sarcoplasmic reticulum. The Ca^{+2}-ATPase transports Ca^{+2} from the cytosol to the interior of the sarcoplasmic reticulum for causing the relaxation of the muscle cells. Release of Ca^{+2} ions from the sarcoplasmic reticulum into the cytosol muscle cells cause contraction of the muscle cells. Sarcoplasmic reticulum tends to concentrate & store Ca^{+2} ions by the help of following 2 types of reservoir proteins-a) Calsequestrin (44,000 daltons M.W., highly acidic protein), which tends to bind up to 43 Ca^{+2} ions with it. b) High affinity Ca^{+2}-binding protein, which binds Ca^{+2} ions & also reduces the concentration of free Ca^{+2} ions inside the sarcoplasmic reticulum vesicles & decreases the amount of energy needed to pump Ca^{+2} ions into it from the cytosol. A Ca^{+2}-pump is a 1,00,000 M.W. polypeptide, forming 80% of integral membrane proteins of sarcoplasmic reticulum. In it hydrolysis of 1 ATP molecule transports 2 Ca^{+2} ions in the counter- transport of 1 Mg^{+2} ion.

3. Proton pump or H+ pump: The lysosomal membrane contains the ATP-dependent proton pump that transports protons from the cytosol into the lumen of the organelle, keeping the interior of lysosomes very acidic (pH 4.5-5.0), the pH of the cytosol is about 7.0.

Proton pumps also occur in mitochondria & chloroplasts, where they participate in the generation of ATP from ADP. They also cause acidification of the mammalian stomach. In the apical membrane of a parietal cell or oxyntic cell (which secrete HCl or H+ Cl-) are located dependent proton pumps. Hydrolysis of ATP is coupled to the transport of H+ ions out of the cell (into stomach lumen). HCl production, thus, involves 3 types of transport proteins: a) anion-exchange protein, b) Chloride (Cl-) permease& c) ATP-dependent proton (H+) pump.

Uniport, Symport & Antiport: Those carrier proteins, which simply transport a single solute from 1 side of the membrane to the other, are called UNIPORT. Others function as coupled transporters, in which the transfer of 1 solute depends on the simultaneous transfer of a 2nd solute, either in the same direction (SYMPORT) or in the opposite direction (ANTIPORT). Both symport & antiport collectively form the co-transport. Most animal cells, e.g., must take up glucose from the extra-cellular fluid, where the concentration of the sugar is relatively high by passive transport through the glucose carrier (D-hexose permease), that operate as the uniports. By contrast, intestinal & kidney cells must take up glucose from the lumen of the intestine & kidney tubules, respectively, where the concentration of the sugar is low. These cells actively transport glucose by symport with Na+ ions whose extra-cellular concentration is very high. The anions exchange permease of Human erythrocytes operates as an antiport to the exchange of Cl- for HCO – (Alberts *et.al*,1989).



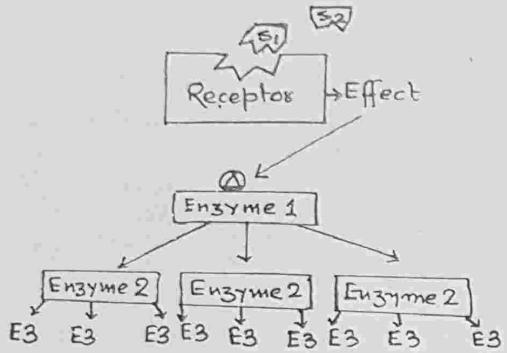
Signal Transduction: The number of different biological signals are large as is the variety of biological responses to these signals, but organisms use just a few evolutionary conserved mechanisms to detect extra-cellular signals & transduce the them into intra-cellular changes. Often the end result of signaling pathway is the phosphorylation of a few specific target cell proteins, which changes their activities & also those of the cells.

Signal transductions are remarkably specific & exquisitely sensitive.

1. Specificity: Specificity is achieved by precise molecular complementarity between the signal & receptor molecules mediated by same kind of weak forces that mediate enzyme-substrate & antigen-antibody interaction. Multi-cellular organisms have an additional level of specificity because the receptor for a given signal, or intra-cellular targets of a given signal pathway are present only in certain cell types.

   Three factors account for the extraordinary sensitivity of the signal transducers: the affinity of receptors for signal molecules, co-operativity in the ligand receptor interaction, & amplification of the signal by enzyme cascades.

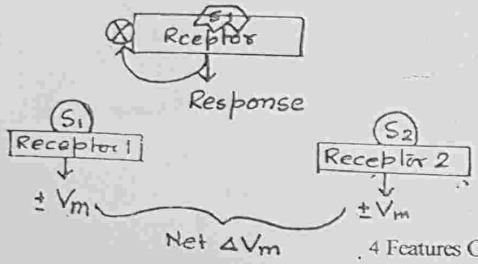
2. Amplification: Amplification by enzyme cascades results when an enzyme associated with a signal receptor is activated & in turn catalyses the activation of many molecules of a 2nd enzyme, each of which activates many molecules of a 3rd enzyme & so on. Such cascades can produce amplification of several orders of magnitude with milli seconds.
3. Desensitization: The sensitivity of receptor system is subjected to modification. When a signal is present continuously, desensitization of the receptor system result. When the stimulus falls below a certain threshold, the system again becomes sensitive.
4. Integration: A final noteworthy feature of signal transduction system is integration, the ability of the system to receive multiple signals & produce an unified response appropriate to the needs of the cell or organism.



Specificity: Signal molecule fits binding site on its complementary receptor, other signals do not fit.

Amplification: When enzymes activate enzymes, the number of affected molecules increases geometrically in an enzyme cascade.

E3 = Enzyme 3



Desensitization: Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.

Integration: When 2 signals have opposite effects on a metabolic characteristic such as the cone of the second messenger X, or the membrane potential Vm, the regulatory outcome results from the integrated input from both receptors.

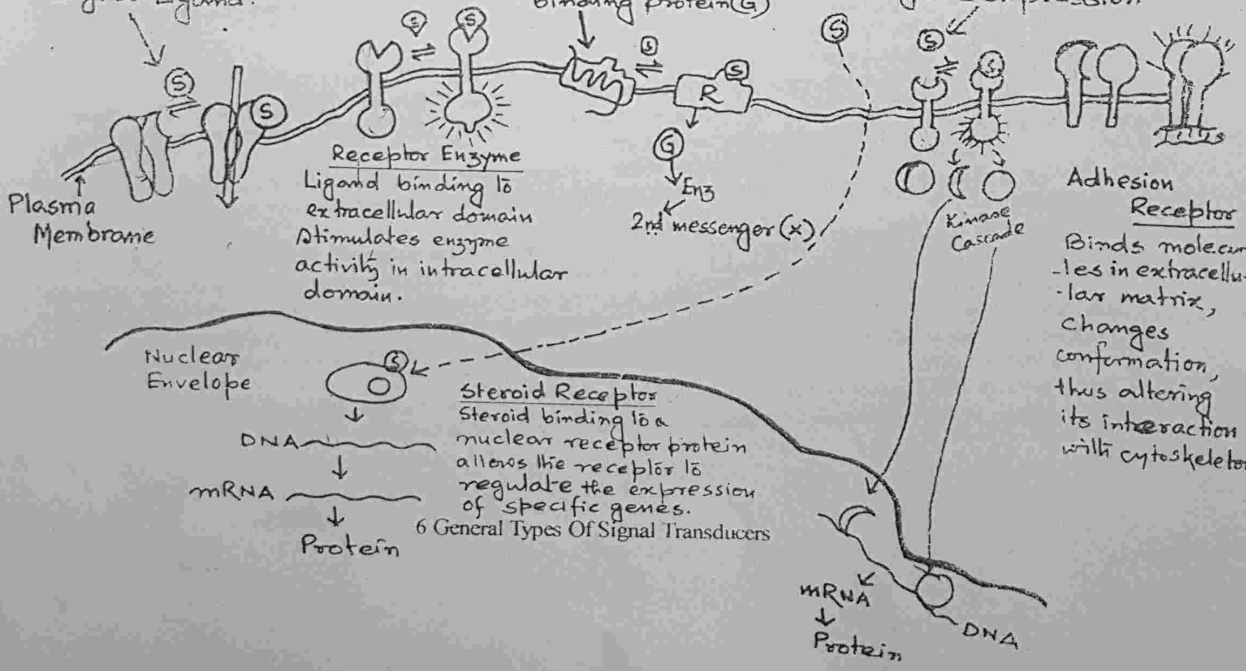
4 Features Of Signal Transduction System

Receptor with no intrinsic Enzyme activity

Interacts with cytosolic Prot. kinase, which activates a gene regulatory protein (Directly or through a Prot. Kinase) changing gene expression

Gated ion channel
Open or closed in response to concentration of Signal Ligand.

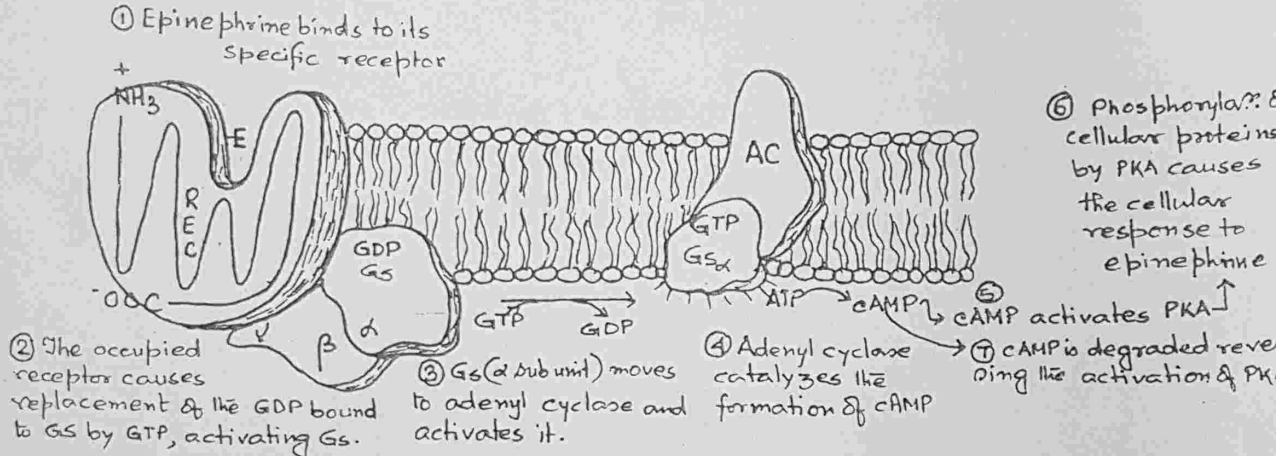
Protein Receptor
External ligand binding to Receptor. Activate an intracellular GTP binding protein(G)



6 General Types Of Signal Transducers

Second Messengers: The term second messenger is a readily diffusible molecule involved in conveying information from an extra-cellular source to the principal target enzymes within the cells. There are 2 principal second messenger in plants- Ca++ & protein kinase.

1. **G-Protein:** G-Proteins, a special group of GTPase super-family, bind with GTP & hydrolyze it. Each of GTPase acts as a molecular switch in which the "on" & "off" are triggered by binding & hydrolysis of GTP, e.g., empty state of GTPase combine with GTP so that GTPase become active. GTP hydrolysis is catalyzed by GTPase activate P-Protein (GAP).



Transduction of the epinephrine signal: Epinephrine (E), Receptor (Rec), Adenylyl Cyclase (AC), G-Protein (Gs).

G-Protein functions as ligand-receptor & interacts in the membrane. During signal transduction ligand-receptor interaction ensures amplification of the correct signal. In plant the involvement of G-Proteins in transduction of blue light, red light, auxin, gibberellin signals & in stomatal aperture regulation, e.g., 1 type GTPase was found to reduce inward K+ current in guard cells.

2. **Ca++/ Calmodulin:** Many of the effects of Ca++ as the secondary messenger, are mediated by Ca++ binding protein, calmodulin which is activated when the concentration of cytosolic Ca++ increases about 0.5m. Calmodulin is a small, highly conserved Ca++ binding protein. It is the primary Ca++ receptor in both plant & animal cells. Calmodulin found in both the cytoplasmic & nuclear compartment & can be attached to the plasmamembrane.

   On binding between Ca++ & calmodulin a Ca++-calmodulin complex is formed. The Ca++/calmodulin then binds to a variety of target proteins including protein kinase. When Ca++/calmodulin bind to the target protein become activated.

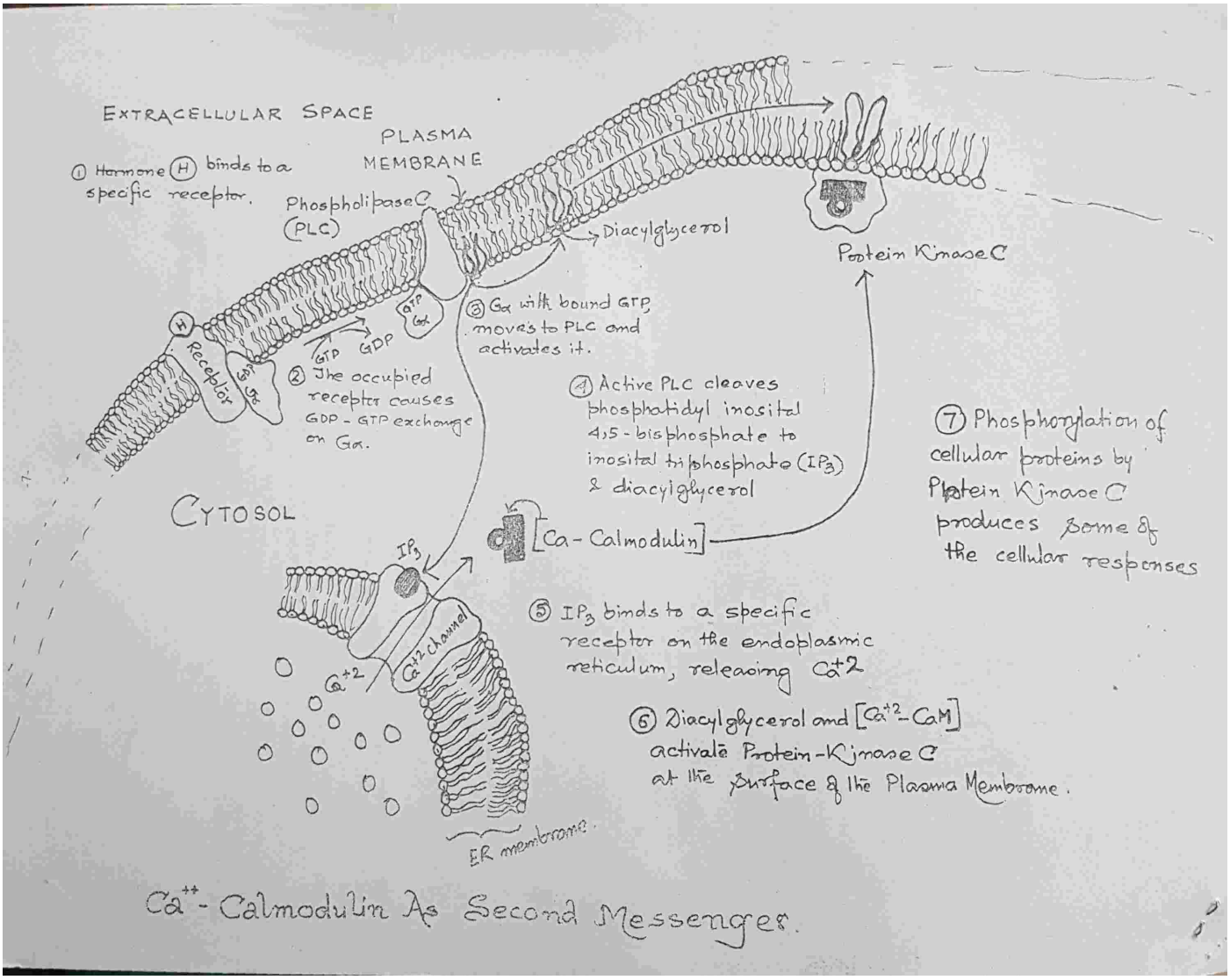
   One of the example of such a Ca++/calmodulin dependent protein kinase is myosin light-chain kinase, which signals actin myosin contraction by phosphorylating 2 of myosin light chains. Other examples of Ca++/calmodulin are the regulation of Adenylyl cyclase (which catalyze the reaction of the formation of camp) & Phosphodiaesterase. Ca++/calmodulin is also important in cytoplasmic streaming, polarized growth of plant body & cell division cycle (at both 'S' & 'M' phases).

3. **Phosphorylated Protein:** Kinase is the protein , which transfers phosphate to the target protein. During the transfer of phosphate, the protein kinase itself become phosphorylated, thus it acts as a phosphorylated protein.

   Protein kinase activity is counter balanced by the action of specific protein phosphatase that remove the phosphate from proteins. In most cases phosphorylation modifies target protein activity. One protein kinase can phosphorylate many hundreds of target proteins, so that weak signals become amplified.

   Several important protein kinases have been identified with regulation metabolic pathways, as well as cell division cycle regulation, growth regulation etc.

   Example- Pollen tube incompatibility is one of the examples of role of protein kinase in signal transduction. During pollination pollen tube send the signals, which received by protein kinase present in the membrane of the stigmatic cells. Now signal transduction occurs, as a result inhibitor is produced, which inhibits pollen tube growth.


EXTRACELLULAR SPACE
PLASMA MEMBRANE
① Hormone (H) binds to a specific receptor.
Phospholipase C (PLC)
Diacylglycerol
Protein Kinase C
H
Receptor
GTP
GDP
② The occupied receptor causes GDP - GTP exchange on Gα.
③ Gα with bound GTP moves to PLC and activates it.
④ Active PLC cleaves phosphatidyl inositol 4,5 - bisphosphate to inositol triphosphate (IP_3) & diacylglycerol
⑦ Phosphorylation of cellular proteins by Protein Kinase C produces some of the cellular responses
CYTOSOL
[Ca - Calmodulin]
IP_3
Ca^{+2} channel
Ca^{+2}
⑤ IP_3 binds to a specific receptor on the endoplasmic reticulum, releasing Ca^{+2}
⑥ Diacylglycerol and [Ca^{+2} - CaM] activate Protein-Kinase C at the surface of the Plasma Membrane.
ER membrane

Ca^{++} - Calmodulin As Second Messenger